

**University of North Carolina, Chapel Hill  
Committee on the Protection of the Rights of Human Subjects (Medical IRB)**

**APPLICATION FOR APPROVAL OF RESEARCH INVOLVING HUMAN SUBJECTS**

**DATE:** June 10, 2005      **IRB STUDY NUMBER (leave blank if new submission):** 03-EPA-393

**TITLE OF STUDY:** Effect of Exposure to Concentrated Ultrafine Ambient Particulate Matter in Young Healthy Adult Subjects (ULTRACON)

**NAME AND DEGREE(S) OF**

**PRINCIPAL INVESTIGATOR:** James M. Samet, Ph.D., Co-PI: Robert B. Devlin, Ph.D.      **DEPT:** EPA

**PID NUMBER OF PRINCIPAL INVESTIGATOR:**

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**NAME AND PHONE NUMBER OF**

**RESEARCH COORDINATOR, IF APPLICABLE:** NA

**NAME OF FUNDING SOURCE:** United States Environmental Protection Agency

**I. Agreements**

**Principal Investigator:**

I certify that each of the above-named co-investigators has accepted his/her role in this study. I agree to a continuing exchange of information with the Committee on the Protection of the Rights of Human Subjects (IRB). I agree to obtain IRB approval before making any changes or additions to the project. I will provide progress reports at least annually, or as requested. I agree to report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. A copy of the consent form will be given to each subject and the signed original will be retained in my files. If the study involves treatment of UNC Hospitals patients, a copy of the consent form will be placed in each subject's medical record.

\_\_\_\_\_  
Signature of Principal Investigator

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature of Faculty Advisor if P.I. trainee or Non-Faculty

\_\_\_\_\_  
Date

**Department Chair of P.I.** (or Vice-Chair if Chair is investigator or otherwise unable to review):

I have reviewed this research study. I believe the research is sound, that the study design and methods are adequate to achieve the study goals, and that there are appropriate resources (financial and otherwise) available to the investigator. I support it, and hereby submit it for further review.

\_\_\_\_\_  
Signature of Department Chair

\_\_\_\_\_  
Department

\_\_\_\_\_  
Date

(rev. 03/19/01)

## II. Summary Checklist

<b>ARE THE FOLLOWING INVOLVED?</b>	<b>YES</b>	<b>NO</b>
Surveys, questionnaires or interviews <i>If research is <u>limited</u> to use of surveys, questionnaires or interviews, Submit <b>Exemption Application Form</b> instead of this application.</i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Existing Patient Records and/or Specimens <i>If research is limited to study of existing medical records and /or samples, Submit <b>Short Form</b> instead of this application.</i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Investigational Drug(s) <b>IND#</b> <i>If "yes", do you intend to use the UNC Hospitals Investigational Drug Service?</i>	<input type="checkbox"/> <input type="checkbox"/>	<input checked="" type="checkbox"/> <input type="checkbox"/>
Approved drugs for "non-FDA-approved" conditions	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Placebo(s)	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Experimental devices, instruments, machines <b>IDE#</b>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Genetic studies on subjects' specimens	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Storage of subjects' specimens for future, as-yet-undesignated research <i>If "yes", see <b>Instructions for Submitting IRB Applications for Research that Includes the Storage of Human Biologic Specimens.</b></i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Fetal tissue	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Videotaping, audiotaping, filming of subjects	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Non-patient volunteers	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Patients as subjects	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Minors (less than 18 years old) <i>If "yes", indicate: <b>Age range</b> to years</i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Do you intend to target your enrollment at:		
-Students or staff as subjects?	<input checked="" type="checkbox"/>	<input type="checkbox"/>
-Non-English-speaking subjects?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
-Decisionally impaired or mentally incompetent subjects?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
-Prisoners, parolees and other convicted offenders as subjects?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
-Pregnant subjects?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Will HIV tests be performed?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Will subjects be studied at off-campus sites?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Is this a multicenter study? <i>If "yes", is UNC-CH the sponsor or coordinating center?</i>	<input type="checkbox"/> <input type="checkbox"/>	<input checked="" type="checkbox"/> <input type="checkbox"/>
Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise <i>If "yes", approval by the Radiation Safety Committee is required.</i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Recombinant DNA or gene transfer to human subjects <i>If "yes", approval by the Biologic Safety Committee is required.</i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Is this an oncology study? <i>If "yes", submit this application directly to the Oncology Protocol Review Committee.</i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Will subjects be studied in the General Clinical Research Center? <i>If "yes", obtain GCRC Addendum from the GCRC and submit complete application (IRB application and Addendum) to the GCRC.</i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>

### III. Required Education in Human Subjects Protection

UNC policy requires that all persons engaged in research involving human subjects must complete training in ethical conduct of research and protection of subjects. This applies to all research, regardless of funding source. For further information, including what options are acceptable in fulfillment of these requirements, see <http://www.med.unc.edu/irb/Education2.htm>

Individuals who have completed training should have been entered into the Human Subjects Training Database maintained by the Office of Research Services (ORS). To print documentation, visit <http://zeppo.admin.unc.edu/isapi/certweb.dll> and enter the names of each individual involved with this research project. Names not returned by the database are not recognized as having satisfied the education requirement. For questions regarding the database, please contact ORS at 962-7757.

**WITH THIS APPLICATION**, please submit the printout from the ORS database, verifying that each individual involved in the research (including faculty, staff, students and outside collaborators, if responsible to this IRB) has satisfied the education requirements.

### IV. Potential Conflict of Interest

The following questions apply to any investigators or study staff involved with industry-sponsored research, and/or their immediate family members (spouse, dependent children, others). Within the past 12 months or the next 12 months, have you or will you:

Receive any form of personal compensation from the Sponsor, including salary, consulting fees, honoraria, royalties, equipment, etc.?

☐ YES ☒ NO

If so, does or will that compensation exceed \$10,000?

☐ YES ☒ NO

Have an ownership interest of any nature in the Sponsor or product under study, including equity, stock options, etc.?

☐ YES ☒ NO

If so, does or will that interest exceed \$10,000 in value?

☐ YES ☒ NO

If so, does that interest represent more than 5% ownership in the Sponsor?

☐ YES ☒ NO

Hold any position with the Sponsor, including officer, director, trustee, consultant, member of advisory board, etc.?

☐ YES ☒ NO

Have an intellectual property interest on any technology or invention used in this study, including patent rights, copyright, etc.?

☐ YES ☒ NO

Have a conflict of interest disclosed through the University's annual evaluation policy that relates to this research study?

☐ YES ☒ NO

If the answer is "YES" to any of the questions above, please include an explanation with this application. As with any changes to the research itself, relationships or interests that develop later should be brought to the IRB's attention for further consideration.

## V. Description of Proposed Research Activity

**Entire application should not usually exceed 5 single-spaced pages using a 12-point font.**

- 1. Purpose and Rationale:** Provide a brief summary of the background information, state the research question(s), and tell why the study is needed. Avoid an extensive literature review.

Numerous epidemiological studies have reported associations between exposure to ambient levels of particulate matter (PM) and various indices of cardiopulmonary morbidity and mortality (See 1 for review). Despite a decade of intensive study, much about the PM health effects problem is still not understood. Ambient PM is a complex mixture that includes bioactive and toxic compounds of natural and anthropogenic origin, several of which have been theorized to be causative or contributory to the adverse effects of PM inhalation. Various physicochemical properties such as particle size and surface area have also been linked to the health effects of PM. Ambient PM can be fractionated into coarse, fine and ultrafine fractions corresponding to particles with mean aerodynamic diameters of  $>2.5$ ,  $>0.1-2.5$ , and  $<0.1$  microns, respectively. One leading hypothesis ascribes the toxicity of PM to particle size, proposing that smaller particles are relatively more potent than larger ones in producing health effects (1-7). Ultrafine particles (UF) have been specifically associated with a worsening of pre-existing pulmonary diseases and have been shown to have a higher acute inflammatory potency than larger particles in animal instillation studies (1, 7). Additionally, a recent study reported that fractional deposition of inhaled UF particles in normal human subjects is relatively high, compared to that for larger particles (8).

The current and proposed National Ambient Air Quality Standard limits on permissible PM levels are set on a mass basis for two particle size fractions, particles greater than 2.5  $\mu\text{m}$  and for those less than 2.5  $\mu\text{m}$  in mean aerodynamic diameter. However, ultrafine particles present a special problem in the regulation of ambient PM. Because of their very small size, ultrafine (UF) particles normally contribute relatively little to the total PM mass; however, these particles are generated in such high numbers by internal combustion engines that UF particles greatly outnumber fine and coarse particles in ambient air. Measurements taken at roadsides in Minnesota indicate the presence of as many as 10 million UF particles/ $\text{cm}^3$  on heavily trafficked roads (9). Thus, it is possible that current and proposed regulation of ambient PM on a mass basis fails to adequately control levels of UF particles. An experimental assessment of the health effects of ultrafine particles in humans is a high priority for US EPA regulatory offices. To this end, **the study described in this protocol will examine health effects of exposure to UF particles concentrated from ambient air in healthy young adult human subjects.**

Controlled exposure of volunteers to PM have been conducted previously. Using a specially designed concentrator capable of concentrating fine ambient air particles by up to 8-fold, researchers in the Human Studies Facility (HSF) have already conducted two such studies (IRB protocols # 95EPA310 and GCRC1541). These studies showed evidence of mild pulmonary inflammation in the bronchoalveolar lavage fluid recovered from young healthy individuals 24 hours following exposure to an average of  $120 \text{ ug}/\text{m}^3$  concentrated Chapel Hill air fine PM for two hours with exercise (10). When healthy elderly subjects were exposed to similar levels of PM, they experienced modest decreases in heart rate variability, as well as changes in blood factors associated with coagulation and clotting (11), (Devlin R.B., unpublished). In addition, in a recently reported study, normal and asthmatic exercising subjects were exposed to synthetic carbon UF particles at concentrations of 2-5 billion particles per liter (approximately  $10-25 \text{ ug}/\text{m}^3$ ) through a respirator mask (8). This study showed a relatively high deposition of UF particles but the subjects have experienced no adverse symptoms, nor have there been significant adverse effects on pulmonary or cardiac function in these subjects. A follow-up study by the same researchers currently exposes subjects to  $50 \text{ ug}/\text{m}^3$  UF carbon particles. In a separate ongoing study at the University of Rochester exposes subjects to as much as  $500 \text{ ug}/\text{m}^3$  of zinc oxide ultrafine particles, with no adverse symptoms reported. However, alterations in leukocyte marker expression and on cardiac repolarization intervals have been documented in this study (Frampton, M., personal communication and (12)).

A new generation of particle concentrator is now available that permits the concentration of particles in the ultrafine to low fine range ( $0.03-0.2 \text{ }\mu\text{m}$  in diameter) (14). A production version of this concentrator has been installed at the EPA HSF for the purpose of conducting exposure studies to concentrated UF particles. The study described in this proposal will expose healthy adult subjects to Chapel Hill ambient air that has been concentrated in its content of UF by a factor of up to 20-fold. As is the case with other ambient PM fractions, ambient UF PM levels vary considerably in concentration and composition over time and location. Hughes and colleagues have reported that the range of mass concentrations for ultrafine particles in the Los Angeles area is  $0.8-1.58 \text{ ug}/\text{m}^3$ . These levels are similar to those obtained

by measurement of UF particles at the inlet to the exposure chamber in the HSF facility in Chapel Hill, which range between 0.3 and 6.7  $\mu\text{g}/\text{m}^3$ , with a mean of 1.6  $\mu\text{g}/\text{m}^3$ .

In terms of particle number, the only metric that can be monitored in real time for UF particles, measurements taken at the HSF and elsewhere in the Triangle area average 5000-7500 UF particles/ $\text{cm}^3$ , with a seasonal variation in the order of 20%. Although the minimal and maximal concentrations in approximately 3000 readings show a 10-fold range in either direction (511 and 49,899 UF particles/ $\text{cm}^3$ , respectively), the standard deviation is less than 100% of the mean, indicating reasonable stability in UF particle numbers over time. Thus, with a concentration factor of 20, the "target" exposure will be between 100,000 and 150,000 UF particles/ $\text{cm}^3$ . In order to allow for temporal variation in ambient levels of UF PM during the exposure, the upper bound exposure limit will not be allowed to exceed 1 million UF particles/ $\text{cm}^3$  averaged over 2 hours. Referring to the mass estimates cited above, the target mass concentration will be approximately 32  $\mu\text{g}/\text{m}^3$  (i.e.,  $20 \times 1.6 \mu\text{g}/\text{m}^3$ ), with a calculated mass limit of approximately 100  $\mu\text{g}/\text{m}^3$ . Note that all mass estimates are presented for illustrations purposes only, as exposures will be monitored on the basis of particle number (see below). Because of potential transient spikes in particle number, the target and exposure maximums will be averaged over the 2 hr exposure period using the procedures delineated below. Please refer to Appendix A for calculations on estimated exposure and deposition doses using varying assumptions.

Occupational limits for exposure to particulate matter in the workplace do not specify particle size. However, exposure to carbon black, essentially a carbonaceous ultrafine particle, is regulated by a proposed threshold limit value (TLV) of 3500  $\mu\text{g}/\text{m}^3$ . Exposure to Diesel soot, a US EPA Class B carcinogen and contributor to the ambient UF burden, is limited by a proposed TLV of 20  $\mu\text{g}/\text{m}^3$ . It is important to note that TLVs are time-weighted averages considered safe for a 40 hr work-week over a working lifetime, while the comparable concentrations to be used in this proposed study involve a single 2 hr exposure (13).

Based on the cardiac repolarization interval effects of exposure to elemental carbon UF observed in the aforementioned study being conducted at Rochester (12), and on epidemiological observations linking cardiovascular effects to ambient UF levels (1), **we hypothesize that exposure of healthy individuals to concentrated UF particles from ambient air will cause detectable hematologic and cardiovascular effects but no evidence of pulmonary inflammation**. This information is expected to be used by EPA regulatory offices in recommending standards for the mitigation of ambient PM health effects.

2. **Subjects:** Specify number, age, gender, ethnicity, and whether healthy volunteers or patients. If patients, specify the disease or condition and indicate how potential subjects will be identified. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided. NIH applications require that women, minorities, and children be included or that their exclusion be justified. If children are involved, refer to "Children as Research Subjects".

Approximately 20 healthy young individuals, aged between 18 and 40, will serve as volunteers. There are no gender or racial restrictions. However, pregnant women or nursing mothers will be excluded from participation as a safety measure since possible effects of UF particles on a fetus or young infants are unknown. All female subjects will be tested for pregnancy at the time of admission into the study and again immediately prior to exposure.

3. **Inclusion/Exclusion criteria:** List required characteristics of potential subjects, and those that preclude enrollment.

Inclusion criteria:

- Age 18-40 years
- Normal resting ECG
- Generally healthy

Exclusion criteria:

- Current smoker or smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year); greater than 5 pack-years during lifetime.
- Oxygen saturation below 96% at the time of physical exam.
- Any chronic medical condition including active pulmonary disease, cardiovascular disease, neurological disease, liver disease, kidney disease, muscular disease, diabetes, other endocrine disease, hematologic/lymphatic disease, immune deficiency or autoimmune disease.

- Any chronic medication usage other than contraceptives, low dose antibiotics for acne, or vitamins/supplements.
- Hepatitis B carriers
- Any significant risk factors for cardiovascular disease (e.g. blood pressure > 140/90).
- Active cancer, history of cancer within the last 5 years, untreated cancer.
- No exposure will be conducted within **4** weeks of a respiratory tract infection.

Subjects with a history of seasonal allergies (hay fever, dust allergies, rhinitis) may be included in the study provided the subject has no respiratory symptoms within 4 weeks of a scheduled exposure; subjects will not be studied during active allergy season. Subjects with a history of childhood asthma may also be included if the subject has had no symptoms within 6 years or has a documented negative methacholine challenge. In addition, individuals unable to discontinue substances that could potentially alter their inflammatory response to PM (e.g. NSAIDs, antioxidants) for at least 2 days prior to exposure will not be allowed to participate.

4. **Full description of the study design, methods and procedures:** Include the type of experimental design; study procedures; sequential description of what will be asked of/done to subjects; assignment of subjects to various arms of the study if applicable; doses, frequency and route of administration of medication and other treatment if applicable; kinds of data to be collected; primary outcome measurements; and follow-up procedures. If the study involves treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls. This section (4) should generally not exceed 2 single-spaced pages using 12-point type.

## EXPERIMENTAL DESIGN

This will be a single-blinded study in which each subject will be exposed to filtered air and to air containing concentrated UF particles (diameter < 0.2 microns) in an exposure chamber. The study will follow a repeated measures design with subjects serving as their own control. Each subject will be exposed to filtered air and to UF particles in a random order, with a minimum of 4 weeks between an air and UF exposures.

### Subject Qualification

**Screening:** Patients will be recruited by the Westat Corporation (see section 12 below). During an initial telephone interview, the volunteers will receive information regarding the study and their eligibility status will be assessed. Volunteers whose responses indicate that they are likely to meet the criteria will be scheduled for an appointment in the Westat recruitment office in the USEPA Human Studies Facility (HSF). At that time the study protocol will be outlined, a screening informed consent form will be signed, and a medical history form completed which contains information on general personal and family medical history.

**Physical exam:** Subjects who are not excluded during the initial screen will be scheduled for a physical examination in the HSF by a licensed physician or nurse practitioner. During this visit subjects will sign an informed consent for a physical and the medical history form completed during screening will be discussed. Subjects will then undergo an abbreviated physical exam including blood sampling (SMA-20 serum chemistry and a complete blood count with differential), 12 lead ECG to screen for baseline cardiac arrhythmias and ST segment and T wave abnormalities, pulse oximetry and spirometry.

**Bronchoscopy physical exam:** A physical exam will also be performed by a physician certified in pulmonary medicine to ensure the subjects are suitable candidates for bronchoscopy. Prior to the examination the subject will be asked to sign a bronchoscopy physical exam informed consent. This examination will include a more thorough assessment of the subject's nares and throat. A spirometry test and/or chest X-ray may be done if indicated by the history and physical.

**Training session:** Those subjects who are not excluded on the basis of the physical exam will undergo a training session to familiarize them with the study protocol, obtain informed consent to complete the study, and to answer any questions they might have regarding their participation in the study. In addition, the exercise level required to reach the target minute ventilation of 20 L/min-m<sup>2</sup> will be established during the training session. This will be accomplished by having the subject exercise for a period of 20-30 min while taking minute ventilation measurements at set intervals. During this visit the subject's first exposure session will also be scheduled.

### Study Procedure Day 1

**Pre-exposure:** On the day of the study, the subject will report to the medical station in the HSF at which time the general health of the subject will be evaluated and the appropriate pre-exposure measurements (HRV, pulmonary function, blood sampling, urine pregnancy test for females) will be completed. Electrodes for telemetry and HRV measurement will be

placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. For HRV measurement, a standard 3-channel, 5-lead configuration will be used in which leads will be placed approximately in the areas above the manubrium of the sternum, at midsternum, and above the xiphoid process and on the right and left mid-axillary line. These leads will be connected to a Holter monitor and will remain in place for approximately 24 hours. Standard telemetry leads will also be placed and removed when the patient leaves for the day. The subject will then be allowed to relax for 30 minutes in a reclined position after which a 10-minute resting HRV measurement will be obtained. Blood samples will then be collected and lung function will be measured following the HRV measurements. The subject will then enter the exposure chamber for filtered air or particle exposure.

**Exposure:** In the chamber, the subject will sit on a cycle ergometer and will undergo a schedule of exercise consisting of 15 minutes of exercise followed by 15 minutes of rest. Exercise levels will be adjusted to a  $V_E$  of 20 L/min- $m^2$ . This cycle will be repeated 4 times during the 2-hour exposure. Telemetry will be monitored continuously throughout the exposure. Subjects will also be monitored continuously by trained personnel for signs of respiratory distress, chest pain, significant cardiac arrhythmias, ataxia, or other signs of distress. The subjects will be aware that they may terminate their exposure at any point should they deem it necessary.

Concentrated UF particles will be generated from ambient Chapel Hill air drawn from above the roof of the HSF and subsequently concentrated approximately 20 fold in order to generate atmospheres containing a "target" concentration of 32 ug (maximum 100 ug/ $m^3$ ) equivalent to a UF particle number target of 100,000-150,000 UF particles/ $cm^3$  (maximum concentration of 1 million/ $cm^3$ ). Ambient air gaseous pollutants will be diluted by a factor of 4 in the process. Particles greater than 2.5um will not enter the concentrator as they will be excluded by a size-selective inlet. The temperature and humidity of the air entering the chamber will be controlled and UF PM-numbers will be measured continuously. During air exposures, the intake air will be filtered to remove 99% or more of the ambient air particles. Particle concentrations will be monitored by particle counters sampling the chamber continuously.

Estimation of Mass of UF Exposure and Exposure Monitoring- The actual concentration of particles that the subjects will be exposed to will be partially dependent on the particle concentration of Chapel Hill air on the day of the study. Therefore, the particle concentration in the chamber may vary from day to day. Concentrations of UF PM in Chapel Hill vary seasonally but levels sampled at the inlet to the exposure facility range between 0.4 and 5.8 ug/ $m^3$  with a mean of 1.6 ug/ $m^3$ . If these particles were concentrated 25 fold, which is the theoretical maximum that can be achieved using the concentrator, we anticipate the average exposure to UF PM mass would be on average 40 ug/ $m^3$  and as high as 145 ug/ $m^3$ . This is comparable to levels of ZnO to which volunteers have been exposed in the Rochester studies.

**Please see appendix for calculations of estimated exposure does and particle number and mass deposition, including upper-bound limits of deposited exposure based on various scenarios.**

Monitoring of UF Exposure Target Concentration and Exposure Limit- At present, real-time UF monitoring instruments can only detect particle number. Currently, there is no device that can report UF particle mass in real-time. **Therefore, particle number, and not mass, will be the metric used for monitoring UF exposure levels in this study. The target dose will be between 100,000 and 150,000 UF particles/ $cm^3$ . Subjects will not be exposed to more 1 million UF particles/ $cm^3$  averaged over the 2 hr exposure.** Per the approved protocol amendment dated March 29, 2004, the first 5 ( five) subjects will be exposed to a maximum limit of 0.5 million particles/ $cm^3$ .

A dedicated particle counter will monitor UF particle counts continuously as the particles enter the exposure chamber from the particle concentrator. The counter is programmed to report counts as 2 minute averages of particle concentration/ $cm^3$ . A computer will keep a cumulative sum of the reported 2 minute averages over the 120 minute exposure period (i.e., 60 averages will be summed by the end of the exposure period). If the sum of the reported averages ever exceeds 85 % of 60 million UF particles/ $cm^3$  during a run, a venting valve on the exposure chamber will be actuated automatically, thus initiating a shut-down procedure leading to removal of the subject from the exposure chamber. Although exposure to UF continues during the 2-3 minute chamber venting time, the flow of particles into the chamber at this time is considerably diminished.

Since the exposures are dependent on the outdoor concentrations of particles, there may be some days in which there are too few particles outdoors for an exposure (e.g. on rainy days). If this happens, a subject who is scheduled to be exposed to particles will be rescheduled and entitled to receive double compensation (\$24/hr) for the time spent at the EPA facility that day.

**Post-exposure:** Immediately following the exposure, the subject will be allowed to rest for 20 minutes and then HRV and T-wave alternans readings will be taken, along with blood sampling, expired breath CO and lung function measurements. The subjects will then be given instructions regarding the holter monitor, NPO status, and the time they are to return to the HSF the next day.

### **Study Procedure Day 2**

The study subjects will return to the HSF to undergo a brief medical evaluation, including spirometry (pulmonary function testing). The subject will then be allowed to relax for 20 minutes in a reclined position after which a 10-minute resting HRV measurement will be obtained, expired breath collected and a blood draw will be taken. Measurements of T-wave alternans will then be carried out after each exposure. This procedure involves the placement of additional electrodes on the subject's chest and is carried out while the subject exercises to a heart rate equal to 70 % of age-adjusted maximal heart rate. Following T-wave alternans measurements, leads will be removed and the subject prepared for bronchoscopy as described below.

### **Study Procedure Days 29+ and 30+**

The procedures for Days 1 and 2 will be repeated no less than 4 weeks later, at which time the subject will be exposed UF PM concentrate or air, depending on the exposure atmosphere in Day 1.

### **OUTCOMES:**

**Lung Function** will be measured before and after exposure. Subjects will perform spirometry, and single breath diffusing capacity (DLCO) on a Sensor Medic Vmax pulmonary function system according to the standard algorithm published by the American Thoracic Society. In addition, regional DLCO and pulmonary capillary blood flow (Qc) will be obtained by the intrabreath technique using the same system.

**Bronchoscopy.** Subjects will undergo standard fiberoptic bronchoscopy with BAL, which will include airway brushing to recover small numbers of airway epithelial cells. Additionally, endobronchial biopsies will be performed on subjects who choose to consent to this procedure for an additional payment (see payment schedule). That is, participation in endobronchial biopsy will be an optional component that will not affect participation in the rest of the protocol. Bronchoscopy will take place approximately 24 hours after each exposure (note that each subject will undergo two bronchoscopies during his/her participation in this study).

BAL measurements will include, but not be limited to, differential cell counts and soluble markers of lung injury and inflammation (e.g. cytokines, prostaglandins, LDH, fibronectin). Epithelial cells removed by brushing will be analyzed for changes in expression of inflammatory genes and other genes indicative of pulmonary injury or response to PM. Endobronchial biopsies, which contain cells and tissues underlying the outer epithelial barrier, will be analyzed by immunohistochemistry for changes in inflammatory cells and markers of activation.

**Procedures for bronchoscopy are described in full detail in a separate approved IRB protocol (IRB # 91EPA304).** Bronchoscopy with BAL and cytology brushes will be performed by a licensed physician board certified in pulmonary medicine and experienced in the use of a fiberoptic bronchoscope and will be assisted by an experienced R.N., N.P. or P.A.. The subject will be monitored during the procedure using telemetry, pulse oximetry, and continuous blood pressure monitoring. The subject's nose and pharynx will then be anesthetized. The subject will gargle approximately 5cc of 4% lidocaine and 2% liquid lidocaine will be dripped into each nostril. This will be followed by insertion of 4% lidocaine jelly into each nostril. The level of anesthesia will be monitored by placing 2 sterile cotton swabs into one of the subject's nostrils. Should the subject feel any discomfort, more lidocaine jelly will be used. The procedure will begin after the subject's nose and oropharynx are adequately anesthetized.

Prior to beginning the procedure, a towel will be placed over the subject's eyes to shield against spraying liquids and a nasal cannula providing supplemental oxygen (up to 4 L/min) will be placed on the side of the nose not used to pass the bronchoscope. A flexible fiberoptic bronchoscope will be passed into the subject's nose and oropharynx and the position will be monitored visually by the physician on a television screen with the use of a camera interfaced to the bronchoscope. Upon nearing the vocal cords, a 1-2% solution of lidocaine will be sprayed through a channel in the bronchoscope to anesthetize them. The bronchoscope will then be passed into the trachea. An injection of 1-2% lidocaine will be made at the main carina and in the proximal right and left bronchi to anesthetize the airway and to minimize the subject's cough reflex during the remainder of the procedure.

BAL will be performed on the side opposite to the side that the brushes will be taken. The bronchoscope will be wedged in a subsegmental bronchus. A volume of up to 270cc of sterile saline per side (one 20cc and up to five 50cc

aliquots) will be injected and immediately aspirated through the channel of the bronchoscope. The lavage fluid aliquots will be collected in polypropylene tubes and kept on ice until processed. After the BAL is complete, the bronchoscope will be repositioned for the endobronchial brushes and biopsies. A sterile cytology brush connected to a long wire will be passed through the channel of the bronchoscope and will be used to obtain surface airway epithelial cells. The brush procedure will be carried out by rubbing the epithelial mucosal surface. After each brushing, the brush is removed and the recovered cells are dislodged from the brush by stirring in a test tube containing sterile tissue culture medium. No more than 6 brushes will be done, each in a different site of the mainstream bronchus.

The subject will be monitored for at least 1 hour by a nurse following the procedure. Chest electrodes will be removed if the ECG tracing is normal, the oxygen cannula will be removed if the oxygen saturation is satisfactory. As a precaution, vital signs will be monitored at least every 30 minutes during recovery. The subject will be discharged by the physician if there are no signs of complications, if the subject has normal vital signs, has a gag reflex, and is able to tolerate oral intake without aspirating. Each subject will be given a pager number and telephone numbers for the medical station and the physician should follow-up be necessary. In addition, subjects will be contacted within 24-48 hours following discharge by a member of the nursing staff.

In cases where participants elect to have forceps biopsies done (for an additional payment), between 1 and 6 scuh biopsies will be taken during each bronchoscopy. Subjects will have to elect forceps biopsies during the first bronchoscopy in order to be eligible to choose forceps biopsies on the second bronchoscopy.

**Heart Rate Variability (HRV)** data will be gathered for 24 hours using a holter monitor. Specific 10 minute epochs to be analyzed for frequency domain variables include times immediately prior to exposure, immediately following exposure, and approximately 24 hours after exposure. Both time and frequency domain variables will be analyzed, as will abnormal responses (e.g. PACs, PVCs, bradycardia, tachycardia).

**Peripheral venous blood samples.** The medical staff will draw approximately 70 mls of blood from each volunteer before exposure, immediately after exposure, and 18 hours after exposure, for a total volume of about 210 mls over a 20 hour period. Samples will be placed into heparinized or citrate-coated tubes and refrigerated until the blood can be processed. Endpoint measurements will include, but not be limited to, the following: markers for specific and non-specific immune responses, coagulation factors, vasoactive factors, and soluble components of PM (e.g. transition metals).

**T Wave Alternans** Changes in cardiac repolarization will be measured using an instrument purchased from Cambridge Heart which is manufactured specifically for this purpose. Twelve lead ECG electrodes will be placed at specific locations on the torso of the volunteer specified in the manual and a baseline reading will be obtained. The volunteer will then begin walking slowly on a treadmill which is part of the TWA instrument. The slope and speed of the treadmill will slowly be increased by computer control according specifications of the modified Bruce Protocol during the next 10 minutes to reach a target heart rate of 70% of the subjects maximum heart rate (calculated from the subject's age). During this time TWA will be calculated and recorded by the computer.

**Analyses of Carboxyhemoglobin and Expired CO** Exposure to ultrafine particles has been found to induce the expression of hemeoxygenase 1 (HO-1) in certain lung cell types and HO-1 activity in lung cells is known to be induced by oxidative stress. A metabolite of HO-1 activity is carbon monoxide (CO) produced through the breakdown of hemoglobin. In order to determine the effect of concentrated UF exposure on endogenous HO-1 activity, expired breath will be collected immediately and approximately 18 hr after exposure to clean air or concentrated UF PM exposure. After taking in a maximal inspiration the subject expires steadily through a mouthpiece (no noseclip) via a high resistance tube which limits flow to ~150 ml per second. After 15-20 seconds (depending on the subject's vital capacity) the remaining flow is collected in a one-liter Mylar bag for 5 seconds (~750 ml). This sample is subsequently analyzed for CO concentration with a carbon monoxide gas analyzer. In order to calculate the portion of the expired CO attributable to the equilibration of blood CO-hemoglobin with alveolar air CO during the period of slow expiration, a 5 ml sample of anticoagulated peripheral venous blood is obtained and its CO-hemoglobin concentration measured spectrophotometrically with an IL-282 CO-oximeter. Healthy non-smokers breathing CO-free air are expected to have blood CO-hemoglobin levels of 0.5-1.0% which will produce equilibrium alveolar CO concentrations of 3-6 ppm. Measured alveolar CO concentrations above the level expected for a given blood CO-hemoglobin level are considered to reflect production of CO in the airways epithelium secondary to hemeoxygenase activity.

5. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable:** Include the number of required visits and approximate duration of each visit.

It is anticipated that the duration of this study will be approximately one year. Subject recruitment and screening is expected to be continuous throughout the study until the intended number of subjects is reached. Scheduling constraints imposed by concurrent studies in the Human Studies Division are expected to limit the rate at which subjects can be exposed to 1 per week. In addition to visits for screening and a qualifying medical examination (which should not exceed 2-3 hours total), each subject will visit the HSF on four occasions, grouped into two pairs. During the first visit, the subject will be exposed to air or CAPs with a duration of about 3-4 hours. The next day the subject will return for bronchoscopy, with a duration of about 4 hours. This process will be repeated no sooner than 4 weeks later.

**6. Where will the subjects be studied?** If off UNC-CH campus, list locations.

All exposures will be carried out at the EPA Human Studies Facility on the UNC campus.

**7. Full description of risks and measures to minimize risks:** Include risk of psychosocial harm (e.g. emotional distress, embarrassment, breach of confidentiality, etc.) economic harm (e.g. loss of insurability) and legal jeopardy (e.g. disclosure of illegal activity) as well as known side effects of study medication, if applicable, and risk of pain and physical injury.

**General measures to minimize the risks:** Medical screening of the potential subjects is designed to exclude those that may be at risk from the study procedures. A physician is on call whenever a subject is undergoing any procedure at the facility. The physician will terminate the procedure at any time if he feels that it would be injurious to the subject's well being to continue. Registered nurses staff a fully stocked medical station and the University of North Carolina Hospital is a short distance from the HSF. On subsequent days after exposure and bronchoscopy, subjects will be urged to contact the medical station or the physician should they experience any of the following symptoms: epistaxis, persistent cough, chest pain, dyspnea, wheezing, hoarseness, or sore throat. Risks associated with specific study procedures are as follows:

- **Pulmonary function tests** (spirometry) are standard non-invasive techniques that are commonly used in studies of pulmonary function on populations of all ages and entail little or no risk to the subject. The intrabreath technique uses acetylene uptake for Qc measurement. Large doses of acetylene are associated with nausea, vomiting, and headache. However, our subjects will be exposed to low concentrations of acetylene (0.3%) for a brief period of time (single inhalation and exhalation), thus we anticipate that the risks of these complications to our subjects will be quite low.

- **ECG and heart rate variability** are standard non-invasive techniques commonly used for heart rate and rhythm analysis and entail little or no risk to the subject. There is the possibility that preparation of the skin for electrode placement and removal may cause skin irritation, itching, or soreness in some subjects.

- **Blood sampling** risks, including pain and hematoma formation, are considered mild and minimal. A licensed RN will take blood samples.

- **Exercise testing** is associated with minimal risk in healthy individuals. It is possible, however, that a previously unidentified preexisting cardiac condition will be uncovered. To minimize this risk, a thorough medical screening will be performed prior to exercise and heart rate and rhythm will be monitored while the subject is exercising. Other side effects of exercise, including occasional muscle soreness, cramps, and general fatigue, are considered temporary and not deemed harmful.

- **T Wave Alternans.** This is a non-invasive technique that should entail no significant risk to the subject. However, there is the possibility that preparation of the skin for electrode placement and removal of electrodes may cause skin irritation, itching, or soreness in some people. In addition, the subjects are asked to walk on a treadmill for 5-10 minutes until a target heart rate equivalent to 70 % of the subjects' maximum heart rate is reached. Since these subjects are young and healthy, it is not thought that this mild exercise will impose a significant risk. **As with all subject procedures, a physician will be in the building and available to respond to any emergency that might result from this test.** Resuscitation equipment, including a fully-stocked crash cart, is available on site for emergencies. The UNC Hospitals Emergency Room is located ½ mile away.

- **Bronchoscopy with BAL and cytology brushes** may be associated with respiratory distress, bleeding, pneumothorax or even death. These risks are explained to the subject in full detail. More than 1200 bronchoscopy procedures have been performed without a serious incident at the Human Studies Division on the UNC-CH campus. Established protocols for bronchoalveolar lavage and brush biopsy ensure that the safety of the subject is given absolute priority. The subject's vital signs, oxygen saturation, and ECG are continuously monitored during the procedure and during recovery. Symptoms which may result in procedure termination include discomfort or anxiety, chest pain, ECG abnormality including tachy- or bradycardia, tachypnea, depressed respiration, moderate bronchospasm, moderate bleeding of the airways, epistaxis, arterial blood saturation less than 96% on supplemental oxygen, or significant changes in blood pressure.

Discomfort of the nose and throat is a common risk of bronchoscopy and will be minimized through the use of lidocaine, which itself presents small risk to the subject. Lidocaine could potentially be absorbed through the nasal

mucosa resulting in systemic effects such as bradycardia, hypotension, urticarial reactions, confusion, lightheadedness, euphoria, tremors, or seizures. To limit these effects, minimum necessary amounts of lidocaine jelly and liquid will be used. Because increased systemic absorption can occur through an inflamed nasal mucosa, subjects with a recent history of upper or lower respiratory tract infection will not be bronchoscoped. Subjects will be continuously monitored for signs and symptoms of lidocaine toxicity.

Epistaxis is caused by trauma to the nose by the bronchoscope. This condition is minor and generally resolves on its own. Small streaks of blood in nasal secretions may be present for up to 12 hours following the procedure. If the bleeding becomes severe, the procedure will be terminated. The subject's anterior nasal passage will be packed with sterile gauze to stop the bleeding. If the bleeding fails to resolve with packing, the subject will be transferred to the NC Memorial Hospital Emergency Room.

Bleeding in the lower airway may occur from trauma caused by the bronchoscope or brushing. The bleeding is typically minor and will typically spontaneously resolve in a matter of minutes. A 1:10,000 or 1:20,000 dilution of epinephrine will be available to stop the bleeding if it does not spontaneously resolve. If epinephrine administration does not resolve the bleeding or if it is sufficiently severe to cause hemoptysis or hemoglobin desaturation, oxygen supplementation will be administered and the subject will be transferred to the NC Memorial Hospital Emergency Room. Epinephrine may be absorbed systemically through the mucosa resulting in transient headache, palpitations, and tachycardia. However, these effects are unlikely to occur due to the small doses used.

There is a small risk of pneumothorax with brushing. Symptoms include dyspnea and chest pain. Subjects will be transferred to the NC Memorial Hospital Emergency Room if a pneumothorax is suspected.

Low-grade fever (38-38.5°C) or pneumonia can occur in subjects undergoing bronchoscopy. The fever typically resolves within 18 hours without treatment or with acetaminophen. In previous studies at the HSF, <1% of subjects undergoing bronchoscopy have reported a fever after the procedure. Risk of pneumonia in the lobe involved in the procedure is also <1%. Symptoms of pneumonia could include fever, dyspnea, persistent or productive cough, and chest pain. The subjects will be asked to contact the physician or nurses at the medical station if symptoms persist or if they experience a fever higher than 38.5°C.

Although the risks are still minimal, forceps biopsies slightly raise the risk of pneumothorax and significant bleeding in the airway.

- **Particle exposure** will occur in young, healthy subjects without pre-existing cardiopulmonary disease. Based on current knowledge, a single exposure to UF air will not have any permanent adverse health effects at the concentrations being used in this experiment. Heart rate, electrocardiogram, and pulse oximetry will be monitored continuously. Subjects will also be monitored for significant respiratory distress or dyspnea, chest pain, significant cardiac arrhythmias, pallor, and ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire exposure session. The investigator or duty physician will end the exposure if the subject is found to be suffering from any adverse effect.

- **Expired Breath Collection** will be conducted in subjects immediately and 18 hrs post exposure to air or UF PM. The collection of breath is carried out by asking the subject to exhale through a narrow orifice into a Mylar bag for a defined period of time. The effort involved in this procedure is less than that involved in a Valsalva maneuver. Consequently, there is a slight risk of transient drop in cardiac output as intrathoracic pressure increases during the expiratory maneuver. Subjects will be seated during collection of expired breath.

8. **Benefits to subjects and/or society:** The possibility of benefit to society should be clearly distinguished from the possibility of benefit to the individual subject, if any. If there is no direct benefit to the individual subject, say so. Do not list monetary payment as a benefit.

Subjects will receive no direct benefit from participating in this study other than receiving a medical examination including blood work, spirometry, and an ECG. Subjects will have full access to their records.

For society, this study will provide new information on the effects of UF air particles on regional lung function, inflammation, and the cardiovascular system. Epidemiology studies currently show an association between UF PM and mortality/morbidity, and some have suggested that the current EPA standard on PM may not be adequate because it does not address UF PM directly. The results from this human study will provide important data that assist the EPA in determining whether UF PM should be considered in the promulgation of PM standards.

9. **Inducements for participation:** If monetary, specify the amount and how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it.

Subjects will receive monetary compensation for their time (approximately \$12/hour) and participation in the study. A subject who is unable to complete the study for voluntary reasons will receive full compensation for his/her participation.

to that point. Subjects who do not complete the study for involuntary reasons will be paid for the entire study. Payment will be made after each segment of the study, unless the subject specifies otherwise.

The following table details the expected compensation for completion of the entire study:

<b>Pre-study Qualifications</b>	
Recruitment Screening	\$15
Physical Exam	\$15
Bronchoscopy Physical Exam	\$20
Training	\$12
Exposure Sessions (2 for 6 hours each)	\$144
(Check-in, pre-testing, exposure and post-testing)	
Blood sampling (6)	\$150
Expired breath samples (6)	\$60
24-hour holter (2)	\$200
Bronchoscopy (2)	\$700
(BAL, cytology brushes, recovery)	
T Wave alternans (6)	\$60
Bonus for Completion of the Study	\$100
<b>Total Compensation</b>	<b>\$1476</b>
<b>Additional compensation</b>	
<b>For forceps biopsies</b>	<b>\$100</b>

In addition, subjects traveling from Durham and Raleigh will be paid an additional \$6 and \$11 per round trip, respectively, and all parking costs will be paid. Since the exposures are somewhat dependent on the outdoor concentrations of particles, there may be some days in which there are too few particles outdoors for an exposure (e.g. if it is raining). If this happens on a day a subject is scheduled to be exposed to particles, the subject will be rescheduled and entitled to receive compensation (\$12/hr) for the time spent at the EPA facility up to 2 consecutive days. Subjects will also receive a nominal reimbursement for bringing in their lunch during exposure days.

**10. Costs to be borne by subjects:** Include clinic fees, diagnostic and laboratory studies, drugs, devices, transportation, all professional fees, etc. If there are no costs to subjects, indicate this.

All procedures and costs directly related to participation in this study will be free of charge to the subjects.

**11. Statistical analysis:** If this is a single-center clinical trial, provide evidence that the sample size is sufficient to achieve the study aims and tell how the data will be analyzed. If a multicenter trial, indicate where and by whom statistical analysis will be performed.

To test our primary hypothesis that UF PM causes negligible or mild lung inflammation and injury we will measure a number of endpoints (e.g. PMN infiltration, cytokine expression, lung function); however the primary pulmonary end point of interest is influx of PMNs into the lung. Several markers of the cardiovascular response to PM will also be measured (e.g. HRV, change in blood coagulation factors, T-wave alternans). Statistical data analyses will consist of paired Student's t-tests for parametric variables and rank sum tests for non-parametric variables.

**12. Methods of recruiting:** Tell how prospective subjects are contacted. If they are UNC Hospital patients, initial contact should be made by their treating physician, or by someone whom the patients know to have legitimate access to their medical records (for example, a clinical director). This may be accomplished by means of a letter from that individual to prospective subjects, requesting the patient's permission to be contacted by the investigator.

Subjects will be recruited for this study by the Westat Corporation, which has recruited subjects for studies at the HSF since 1998. The manner in which this will be done is identical to that of past EPA studies and specific recruitment procedures are described in the subject recruitment protocol on file with the UNC Committee for the Protection of the Rights of Human Subjects. The population targeted will be young healthy residents of the Triangle area. *Every effort will be made to recruit women and members of racial minority groups into this study* Volunteers will be asked to call the recruitment office. During the telephone interview, the volunteers will receive information regarding the study and their eligibility status will be assessed. Volunteers whose responses indicate that they are likely to meet the criteria will be scheduled for an appointment in the Westat recruitment office in the Human Studies Facility. At that time the entire study protocol will be outlined, and a medical history form will be administered.

**13. How will informed consent be obtained?** Describe the process. When the consent of a legally authorized representative is substituted for consent of the adult subject, explain why this is necessary. If non-English-speaking subjects will be enrolled, a consent form should be prepared in their foreign language. Someone who is fluent in the subjects' language must be available to interpret.

Before being selected as subjects, all volunteers will be required to read and sign a form asserting that they have read and understood the following: 1) Subject participation is strictly voluntary, 2) The purpose of the study, 3) The nature and extent of subject participation, 4) The subject's rights to withdraw at any time, 5) The subject's right to privacy, 6) The risks associated with participation, 7) The method and schedule of compensation, and 8) The limits of the University and PI's liability.

One of the PIs or a designated co-PI will briefly describe the study and answer any questions that each subject might have regarding his/her participation, the safety of the procedures, issues related to payment, etc. The PI will then review the contents of the consent form before he and the subject sign it. Subjects will have the opportunity to ask questions at any time during the study by contacting one of the PIs and/or the medical staff. Subjects will be asked to sign a written informed consent form after all of their questions and concerns have been addressed. One signed copy of the written informed consent will be given to the subject while the investigators will retain the original.

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## APPENDIX A

### Exposure Dose Calculations

Assuming 10 L/min at rest and 35 L/min (calculated from a  $V_e$  of 20 L/min- $m^2$ ), then the average ventilation rate is 22.5 L/min. Over 120 min exposure period = 2700 L of air respired.

With a target concentration of  $1 \times 10^5$  particles/cm<sup>3</sup> =  $1 \times 10^8$  particles/L, we get

(2700 L respired) ( $1 \times 10^8$  particles/L) =  $2.7 \times 10^{11}$  (270 billion) particles respired during the 2-hour exposure.

The range for the target is  $\pm 1.5$  x, so the actual value could be  $4.0 \times 10^{11}$  (400 billion) particles respired during the 2-hour exposure.

The concentration limit in the chamber is  $1 \times 10^6$  particles/cm<sup>3</sup>, so the worst case scenario is  $2.7 \times 10^{12}$  (2.7 trillion) particles respired during the 2-hour exposure.

### A Cross-Check

As described earlier in this protocol, the estimated average ambient UF particle number used in these calculations is 5,000/cm<sup>3</sup>, while the mass of UF particles is estimated to average 1.6 ug/m<sup>3</sup>. These numbers are derived from independent measurements using entirely different methodologies. If both of these values are reasonably representative of UF particles in the ambient air, it should be possible to use them to calculate a particle size that falls within a reasonable UF particle range.

Assuming a mass density of 2.5 g/cm<sup>3</sup>, if 5,000 UF/cm<sup>3</sup> is equal to 1.6 ug/m<sup>3</sup>, then the calculated spherical particle size is 62.5 nm, which is well within the UF particle range.

### Deposition Dose Calculations

#### *Particle Number*

If we predict a 50 % deposition, then the target exposure will result in  $1.4\text{-}2.0 \times 10^{11}$  (140-200 billion) particles being deposited, with a worst-case scenario of  $1.4 \times 10^{12}$  particles being deposited over 2 hours.

If we assume that all of the particles are about 10 nm, we can assume an 80% deposition and get  $2.2\text{-}3.2 \times 10^{11}$  (220-320 billion) particles deposited, and an absolute worst case scenario of  $2.2 \times 10^{12}$  (2.2 trillion) particles deposited over 2 hours.

#### *Particle Mass*

Assuming all of the particles are 10 nm in diameter, using  $\frac{4}{3}\pi r^3$ , we get a volume per particle of  $5.24 \times 10^{-19}$  cm<sup>3</sup>, and assume a density of 2.5 g/cm<sup>3</sup>, we get a mass of  $1.31 \times 10^{-18}$  g for each particle. For 2 trillion ( $2 \times 10^{12}$ ) particles deposited, that gives a mass of  $2.6 \times 10^{-6}$  g, or 2.6 ug, deposited over 2 hours.

If we assume that all of the particles are 100 nm in diameter, each particle is  $5.24 \times 10^{-16}$  cm<sup>3</sup> and weighs  $1.31 \times 10^{-15}$  g. A trillion of them would weigh  $1.3 \times 10^{-3}$  g, or 1.3 mg deposited over 2 hours.

Therefore, the estimated mass deposition of particles could range between 1.3 ug and 1.3 mg during the 2 hour exposure period. Because the particle mass is a cubic function of the particle diameter, the size distribution of the particles will have a large effect on the mass of particles deposited. However, we feel that these numbers represent lower- and upper-bound estimates of deposited exposure mass assuming extreme scenarios.

For comparison, the Rochester ZnO studies have been performed using concentrations in excess of  $500 \text{ ug/m}^3$ , which would translate into an exposure of 1200 ug over 2 hours. In addition, the occupational limit to carbon black is  $3.5 \text{ mg/m}^3$ , which would mean 8.4 mg total exposure over 2 hours in this protocol.